BIOLOGICAL TECHNIQUES

A Laboratory Manual

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Gas chromatography (GC) analysis

The FAME samples were analyzed by gas chromatograph (Shimadzu, GC2014, Japan) with flame ionization detector (FID). One microlitre of each sample was injected into FAMEWAX column (Restek, USA) (30 m×32 mm ID×25 µm film thickness). The temperature program was as follows: initial 140 °C with 5 min hold; ramp 2 °C/min to 230 °C with a 5 min hold. Column flow was set at 22.2 mL/min. The instrument condition was as follows: carrier gas nitrogen; FID set at 260 °C, and split ratio of 10:1. The run time for a single sample was 55 min. Each sample was analyzed in triplicates, and FAME identification was done by comparison with standard certificate, Supelco FAME mix C4 – C24 (Bellefonte, PA, USA).

24. GAS CHROMATOGRAPHY – MASS SPECTROMETRY: BASIC PRINCIPLE, TECHNIQUE AND APPLICATIONS

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Introduction

Gas chromatograph (GC) is certainly one of the best tools available for screening, identification and quantification of many groups of non-polar and semi-polar chemicals. Greater separation power of GC in combination with good identification characteristics of mass spectrometry (MS) makes GC-MS an important tool in the analysis of ultratrace levels of chemicals that occur in complex matrices such as water, sediment, plant and animal tissues, microbial fermentation products, etc. In Biological Sciences, it is used for metabolic profiling, analysis of drugs and metabolites in blood, urine, tissue samples, screening of bioactive compounds in plants, and for identifying metabolic disorders, etc. While in Environmental Sciences, GC-MS is used for monitoring of conventional and emerging organic pollutants like pesticides, industrial chemicals, petroleum hydrocarbons, flame-retardants, plasticizers, pharmaceuticals and personal care products (PPCPs), etc.

Principle

In general, GC-MS is composed of two major building blocks; the Gas Chromatograph and the Mass Spectrometer connected to each other via an interface. The gas chromatographic separation of compounds takes place in the column and depends on the nature of compound, column's dimensions (length, diameter, film thickness) as

well as the column packed material (e.g. 5% phenyl polysiloxane) etc. The difference in the physio-chemical properties (e.g. boiling point and polarity) between different molecules in a mixture is responsible for the separation of molecules depending on interactions with the stationary phase in the column. Each molecule takes different amount of time (called the retention time) to elute from the gas chromatograph (column) and this allows the downstream mass spectrometer to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge (m/z) ratio(Hubschmann, 2015). Fig. 1 shows the process of separation of environmental contaminants by GC-MS.

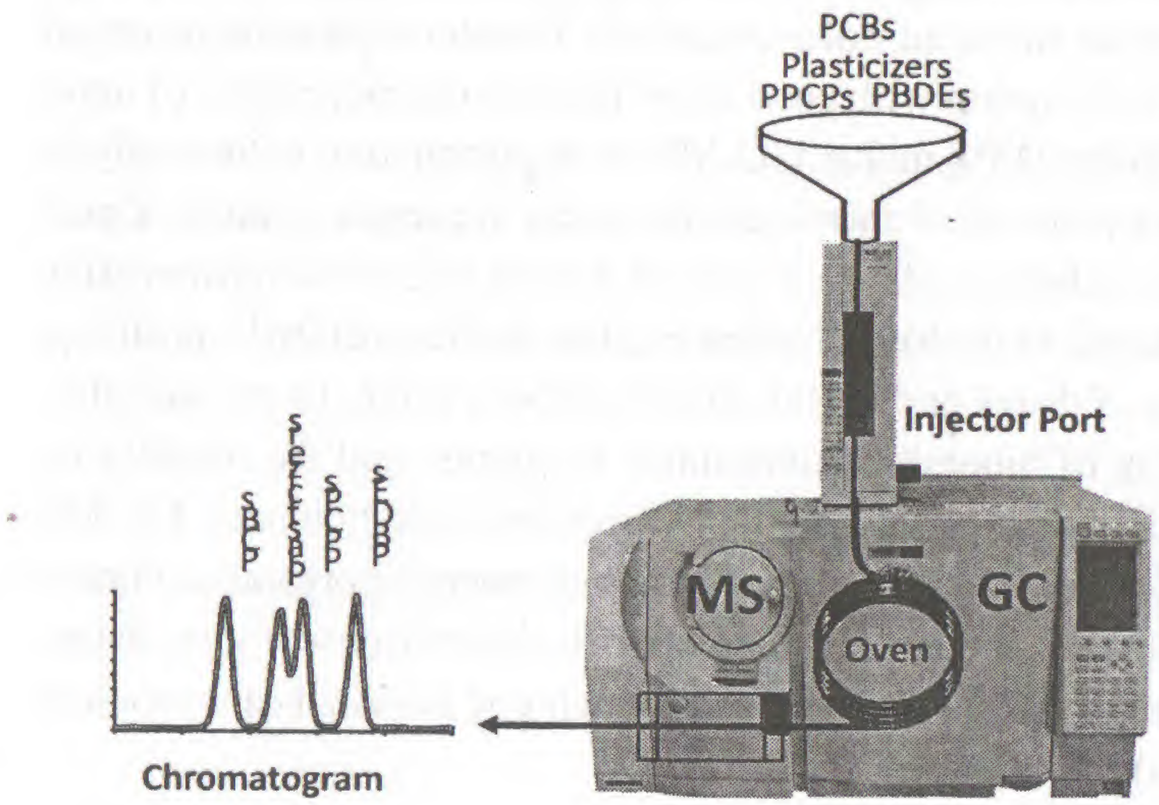

Fig. 1 Separation of mixture of compounds by GC-MS

Working Procedure

Initially, Sample (analytes) dissolved in non-polar organic solventis injected, via syringe, through a rubber septum into the heated injection port, where the sample is volatilized. The most common

injector is the split/splitless injector which can operate in either the split or splitless mode.

In the split injection mode, only a fraction of the vaporized sample is transferred onto the column. The remainder of the vaporized sample is removed from the injection port via the split vent linein a set ratio known as the split ratio. In a splitless system, total injected volume of the sample extract is transferred into the column. Here, the advantage is that a larger amount of sample is introduced to the column. However, a split system is preferred when the detector is sensitive to trace amounts of analyte and there is concern about overloading of column (Hubschmann, 2015).

The injection port ends in a column, which is placed inside the temperature-controlled oven designed to hold and heat the column according to the temperature programme. Carrier gas, usually either nitrogen, helium, or hydrogen, is used to carry the injected sample down the column where the separation takes place. Further,the sample goes into the GC-MS interface.

The interface acts as a transfer line to carry the pressurized GC output into the evacuated ion source of the mass spectrometer. The entire MS system works under intense vacuum condition and it has three basic sections: an ionization chamber, the analyzer, and the ion detector (Fig. 2).

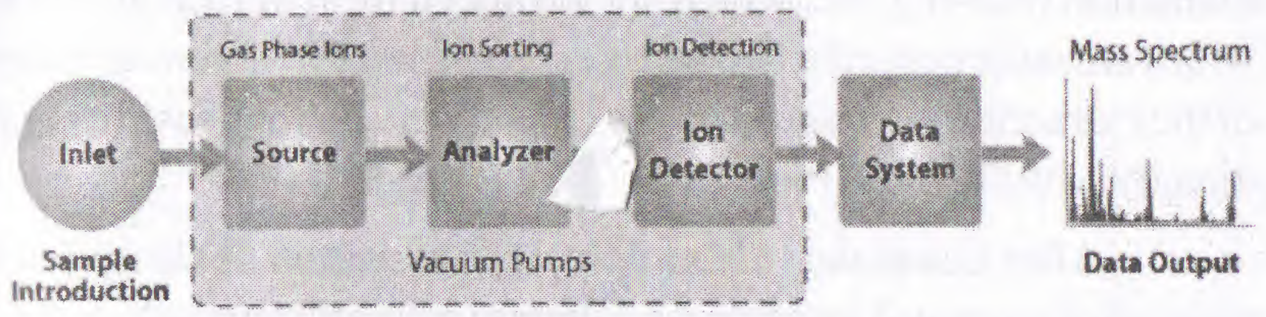

Fig. 2 Schematic representation of mass spectrometer

Interface transfers the output of GC into the mass spectrometer where molecules are ionized and accelerated. The accelerated ions are further separated by its m/z mass in mass analyzer

via electromagnetic deflection produced by quadrupole mass analyzer. The separated mass ions pass through the quadrupole selectively based on the applied dc and RF into the detector.Finally,on entering the detector, the ions are deflected onto a cascade plate where the signal is multiplied and sent to the data system to generate mass spectrum. The summed raw signal can be plotted against time as a total-ion chromatogram (TIC) or a single-ion m/z can be extracted and plotted against time as a single-ion chromatogram (SIC) (McMaster, 2008).

Sample Preparation Techniques

Sample preparation is an important prerequisite for GC-MS analysis. There are number of separation techniques available based on the matrix and extraction condition. The most common approach for the extraction of compounds from aqueous samples is liquid–liquid extraction (LLE).The principal of liquid–liquid extraction is that a sample is distributed or partitioned between two immiscible liquids or phases in which the compound andmatrix have different solubility.Solid phase extraction (SPE) is advanced sample preparation method used for isolation, enrichment and/or clean-up of components of interest from aqueous samples.This method uses less solvent, fast and cost effective. In case of solid samples, soxhlet extraction and ultrasonication are commonly used. Modern development in extraction techniques has resulted in emergence of microextraction techniques such as liquid-liquid microextraction (LLME), solid phase micro extraction (SPME), etc. which are advanced form of LLE and SPE which are more cost effective and user (eco)friendly. Moreover, some of the extraction techniques such as SPME can be connected online directly with GC-MS (Pietrogrande and Basaglia, 2007).

After extracting the sample, it is subjected to cleanup for removal of unwanted interference to improve the efficiency of analysis (Fig. 3). Mainly, column packed with materials such as silica gel, florisil and alumina is used for cleanup process. Further, the sample is condensed and optionally derivatized for semi-volatile analytes to make them more volatile. This step increases the detector response for

semi-volatile compounds. Silylation, alkylation and acylation are common derivatizing methods (Fig. 3). Most commonly used silyl derivatizing reagents includes N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA). Finally, the sample extract of 1 µLis subjected for GC-MS analysis. Sample can be subjected for qualitative analysis in scan mode where as selected ion monitoring (SIM) mode is used for quantitative analysis (Dean, 2009).A representative chromatogram for paraben preservatives in breast cancer tissueis givenin Fig. 4.

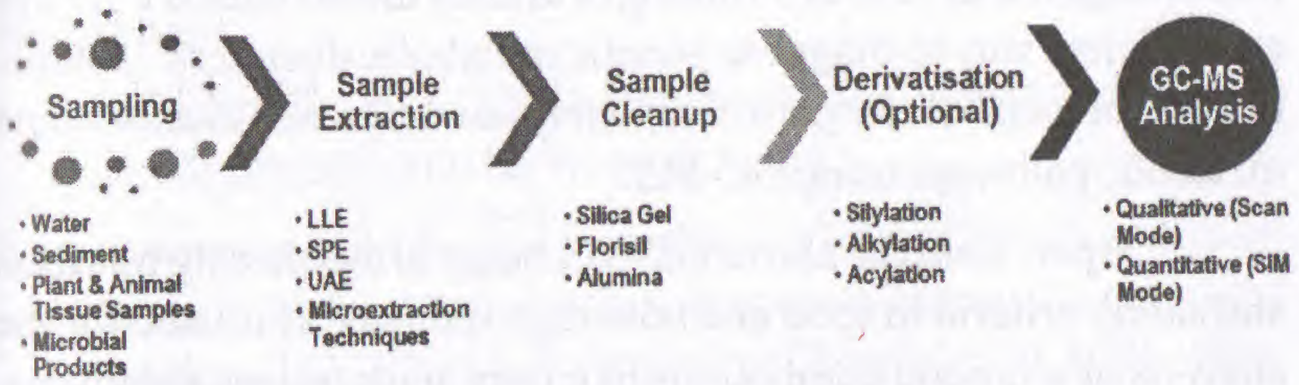

Fig. 3 Analytical process involved in GC-MS analysis

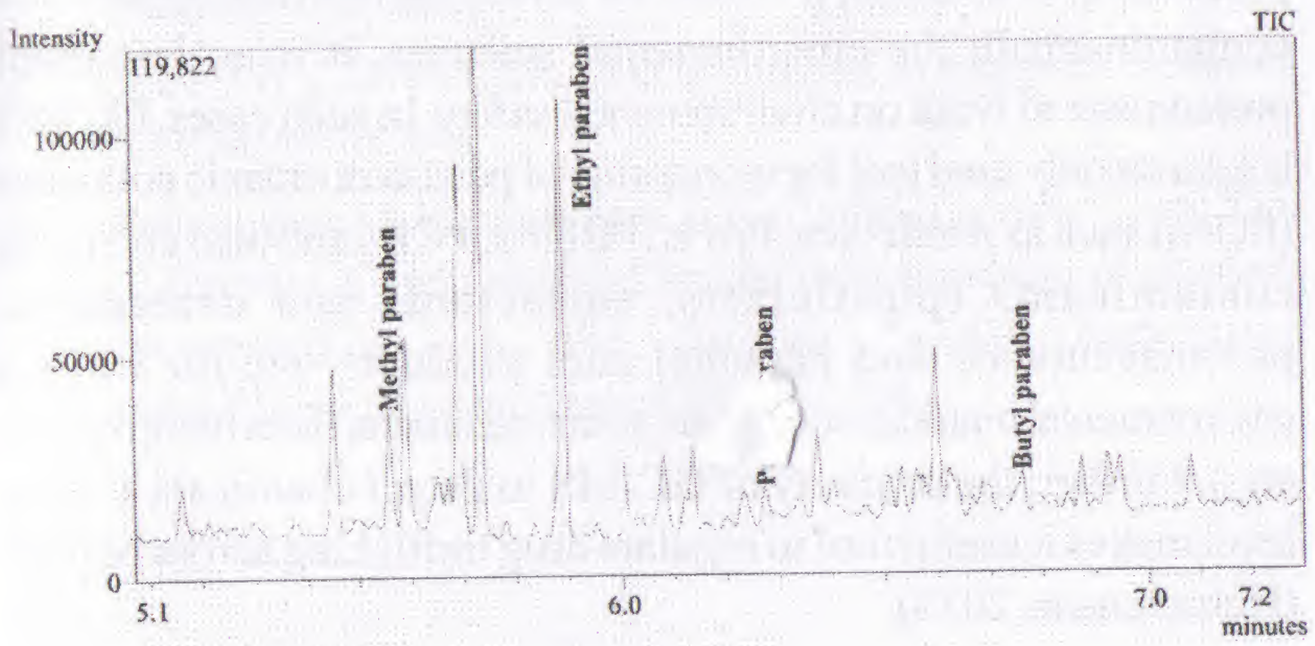

Fig. 4 GC-MS Chromatogram showing parabens in breast cancer tissue

Applications of GC-MS

GC-MS has applications in various fields due to its versatility. In biological sciences, it is used for analysis of blood, urine and other tissue samples for the presence of drug metabolites, illicit drugs; fatty acid profiling of microbes,profiling of bioactive compounds in plants, etc. In case of health care industry, GC-MS is extensively used in pharma sector for quality controlat various stages of drug development and production. In disease diagnosis, role of GC-MS is gaining importance day by day. Metabolic diseases are now detected by screening the specific metabolites/markers responsible for the disease (Shanmugam et al., 2010). Profiling of urinary metabolites is effective and efficient way to diagnose genetic metabolic disorders. Isotopic labeling of metabolitesopens completely new approach to understand metabolic pathways using GC-MS.

Apart from the above GC-MS helps in monitoring nutrition and safety criteria in food and beverage industry. It is used for the analysis of aromatic compounds like fatty acids, esters,aldehydes, alcohols, terpenes, etc. present natively or formed while food processing. In addition, it is used for screening food adulterants and contaminants.In the environmental sciences, it is an important prerequisite to work on environmental safety. In such cases, GC-MS is a commonly used tool for monitoring of persistent organic pollutants (POPs) such as pesticides, PAHs, PBDEs, PCBs and also emerging contaminants (plasticizers, surfactants and detergents, pharmaceuticals and personal care products, etc.)in various environmental matrices such as air, water, sediment, biota (fish, mussel, etc.). Further, the property of GC-MS to detect chemicals at trace level makes it useful tool to regulate drug trafficking across borders (Hubschmann, 2015).

Conclusion

Gas Chromatography – Mass Spectrometry is one of the highly useful techniques, which ensures good separation with high selectivity and low-detection limits of analytes. This makes it as an

ideal tool for application in analytical fields including biological and environmental research. This versatile analytical technique could also be explored for better prospects degradative/intermediate compounds of natural or anthropogenic compounds.

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